

CLAIMS

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DETAILED DESCRIPTION
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1. A method of specifically amplifying desired regions of nucleic acid from a sample containing nucleic acid comprising:

- (a) providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;
- (b) providing a plurality of second PCR primers, each second primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; and then
- (c) amplifying the nucleic acid present in the sample via the PCR using the plurality of first PCR primers and the plurality of second PCR primers; whereby a subset of the plurality of first primers binds to the consensus sequence of interest substantially wherever it occurs in the sample, and a subset of the plurality of second primers binds to the sample at locations removed from the first primers such that DNA regions flanked by the first primer and the second primer are specifically amplified.

- 2. The method of Claim 1, wherein the sample containing nucleic acid contains genomic DNA.
- 3. The method of Claim 1, wherein the sample containing nucleic acid contains eukaryotic genomic DNA.

4. The method of Claim 1, wherein the sample containing nucleic acid contains human genomic DNA.

5. The method of Claim 1, wherein the sample containing nucleic acid contains prokaryotic DNA.

6. The method of Claim 1, wherein the sample containing nucleic acid contains DNA selected from the group consisting of ~~cloned~~ genomic DNA, a subgenomic region of DNA, a chromosome, and a ~~subchromosomal~~ region.

7. The method of Claim 1, wherein the sample containing nucleic acid contains RNA.

8. The method of Claim 1, wherein in step (a) is provided a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence complementary to a consensus sequence selected from the group consisting of a promoter sequence, a 3' splice sequence, a 5' splice sequence, an Alu repeat, a tandem repeat, poly-A site, a lariat signal, a microsatellite sequence, and a homeobox sequence.

9. The method of Claim 1, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.

10. The method of Claim 1, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of over 50%, under 50%, and about 50%, and in step (b) is provided a plurality of

second primers having a G + C content selected from the group consisting of over 50%, under 50%, and about 50%.

11. The method of Claim 1, further comprising step (d): incorporating the amplified fragments of step (c) into a library.

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A method of specifically amplifying exons from a sample of DNA comprising:

- (a) providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of a 3' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;
- (b) providing a plurality of second PCR primers, each second primer having a region of fixed nucleotide sequence reversely complementary to a consensus sequence of a 5' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; and then
- (c) amplifying the genomic DNA via the PCR using the plurality of first PCR primers and the plurality of second PCR primers; whereby a subset of the plurality first primers binds to a sequence reversely complementary to the 3' splice consensus sequence substantially wherever it occurs in the sample, and a subset of the plurality of second primers binds to the 5' splice consensus sequence substantially wherever it occurs in the sample, such that exons flanked by the first primer and the second primer are specifically amplified from the sample.

13. The method of ~~Claim 12~~, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.

14. The method of ~~Claim 12~~, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of cover 50%, under 50%, and at 50%, and in step (b) is provided a plurality of second primers having a G+C content selected from the group consisting of cover 50%, under 50%, and at 50%.

15. The method of ~~Claim 12~~, further comprising step (d): incorporating the amplified fragments of step (c) into a library.

16. The method of ~~Claim 12~~, wherein genomic DNA is specifically amplified.

17. The method of ~~Claim 12~~, wherein human genomic DNA is specifically amplified.

18. The method of ~~Claim 12~~, wherein DNA selected from the group consisting of cloned genomic DNA, a subgenomic region of DNA, a chromosome, and a subchromosomal region is used as the sample DNA.

19. A method of specifically amplifying regions flanking a consensus sequence in a sample of nucleic acid of totally or partially unknown sequence comprising:
(a) providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide

sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;

10 (b) providing a plurality of second PCR primers, each second primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; then

15 (c) amplifying the nucleic acid present in the sample via the PCR using the plurality of first PCR primers and the plurality of second PCR primers; whereby a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the sample, and a subset of the plurality of second primers binds to the sample at locations removed from the first primers such that DNA regions flanked by the first primer and the second primer are specifically amplified; then

20 (d) incorporating the amplified nucleic acid of step (c) into a library;

25 (e) sequencing a portion of amplified nucleic acid from a particular clone from the library of step (d) and providing a third PCR primer of unique sequence which will prime PCR amplification from the sequenced portion of DNA;

30 (f) providing a plurality of fourth PCR primers, each fourth primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; and then

(g) amplifying the nucleic acid present in the sample via the PCR using the third PCR primer and the plurality of fourth PCR primers; whereby the third primer binds to the sequenced portion of nucleic acid from step (e), and a subset of the plurality of fourth primers binds

to the sample at locations removed from the third primers such that DNA regions flanked by the third primer and the fourth primer are specifically amplified.

20. The method of Claim 19, wherein the sample containing nucleic acid contains genomic DNA.
21. The method of Claim 19, wherein the sample containing nucleic acid contains eukaryotic genomic DNA.
22. The method of Claim 19, wherein the sample containing nucleic acid contains human genomic DNA.
23. The method of Claim 19, wherein the sample containing nucleic acid contains prokaryotic DNA.
24. The method of Claim 19, wherein the sample containing nucleic acid contains DNA selected from the group consisting of cloned genomic DNA, a subgenomic region of DNA, a chromosome, and a subchromosomal region.
25. The method of Claim 19, wherein the sample containing nucleic acid contains RNA.
26. The method of Claim 19, wherein in step (a) is provided a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence selected from the group consisting of a promoter sequence, a 3' splice sequence, a 5' splice sequence, an Alu repeat, a tandem repeat, poly-A site, a lariat signal, a microsatellite, and a homeobox sequence.

27. ~~The method of Claim 19, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.~~

28. The method of Claim 19, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of cover 50%, under 50%, and at 50%, and in step (b) is provided a plurality of second primers having a GC content selected from the group consisting of cover 50%, under 50%, and at 50%.

29. The method of Claim 19, further comprising step (h): incorporating the specifically amplified fragments of step (g) into a library.